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(54) The use of autologous promoters to express gene products in bordetella.

(57) Autologous promoters are used to effect expression of gene products in pertissis strains. Hybrid pertussis genes are constructed comprising a structural pertussis gene fused at an ATG codon to a native but autologous pertussis promoter. B. pertussis, transformed by the hybrid gene by insertion into the chromosome of the organism by homologous recombination at specific loci, effects expresion of the antigenic protein for which the structural gene codes at a production rate different from that achieved for the homologous gene. Specific strains and plasmids are described. The technique is applicable to a wide variety of gene products and organisms.

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FIELD OF INVENTION

The present invention relates to a nov l approach to vary the production of g ne products, particularly protein antigens, particularly in species of Bordetella, by changing the promoters of the gene coding for the respective proteins and altering their level of expression.

BACKGROUND TO THE INVENTION

The bacterial species Bordetella comprises B. pertussis, B. parapertussis, B. bronchiseptica, and B. avium. The first two microorganisms are human pathogens, while the latter two are generally restricted to non-human hosts. B. pertussis and B. parapertussis cause the disease whooping cough with the former generating more severe symptoms. The disease, or vaccination against the disease (using an inactivated whole cell vaccine), elicits antibodies agains several antigens, typically pertussis toxin (PT), filamentous haemagglutinin (FHA), agglutinogens or fimbriae and the 69kDa outer membrane protein or pertactin. These proteins represent the major immunogens that may be included, individually or in combination, in any vaccins used to protect against the disease, whether it be the inactivated whole-cell vaccine or a defined component vaccine. Therefore, th efficient expression of these antigens from the vaccine strain is crucial.

During the production of vaccine antigens by fermentation, it has been observed that FHA is secreted at approximately 7 and 10 times tge molar level of pertactin and PT, respectively. While protein structural complexity and secretion efficiencies may be important factors influencing antigen yields, the level of expression of B. pertussis antigen genes also may be influenced by the relative strength and the regulation of their respective promoters. Thus, it may be possible to optimize antigen production by substituting autologous promoters, which may either increase or decrease the yield of selected antigens. The resulting B. pertussis strains would be more economical and better immunogens to use directly in whole-cell vaccines. In addition, promoter interchange also may represent a mean to enhance fermentation and downstream processing efficiencies for component vaccines by altering the kinetics of production and yields of specific antigens.

Along with other genes, the pertussis toxin operon (TOX), the FHA operon, and the pertactin gene (PRN) are all positively regulated by the Bordetella virulence regulating gene (Bvg), formerly known as VIR. The nucleotide sequences of the TOX, FHA, and PRN structural genes and their prometers have been established and the corresponding protein sequences derived (see below). For the TOX operon, th Bvg responsive region of the promoter has been mapped to a position -170 bp from the start of transcription. The corresponding regulatory regions of the other genes hav not yet been determined.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a novel method to alter protein expression in Bordetella species by substituting the promoter of one structural gene by that of another. It is possible to increase or decrease protein expression using this strategy as well as achieving synchronicity or asynchronicity of production.

Accordingly, in one aspect, the present invention provides a hybrid pertussis gene, comprising a structural pertussis gene fused at an ATG start codon to a native but autologous pertussis promoter.

Preferably, specific combinations of structural genes and promoters are provided, including the TOX promoter in combination with the FHA or PRN structural gene; the FHA promoter in combination with the TOX or PRN structural gene; and the PRN promoter in combination with the TOX or FHA structural gene.

The present invention further provides a strain of Bordetella, particularly Bordetella pertussis, which has been transformed by the hybrid gene or a multiple of such hybrid genes and is capable of expression of a gene product of the structural pertussis gene. This transformed strain produces, upon culture of the transformed strain, the gene product at a yield of production which is altered from the yield of production achieved by the same bordetella strain transformed by a homologous gene comprising a structural pertussis gene fused at an ATG start codon to its own natural pertussis promoter.

Specific transformants of specific B. pertussis strains are described in the disclosure which follows, in particular those identified as B. pertussis strains Nos. 1290-4, 390-59, 590-4 and 890-49. In addition, non-transformed B. pertussis strains Nos. 390-101 and 1090-108-3, from which the FHA operon and the PRN gene respectively have been removed, form other aspects of the invention. The invention further includes the plasmids useful in effecting transformation of B. pertussis strains.

The gene products, namely the antigenic proteins, produced by culturing the transformed cells are useful in component vaccines against B. pertussis.

An aspect of the invention allows for the easier downstream separation of the resulting proteins, which makes the production of a component vaccine more economical. A further aspect of th invention allows for the preparation of a whole-cell pertussis vaccine in which, because of improved gen expression, antigenic proteins are better distributed.

The technique described herein for expression of antigenic pertussis proteins from transformed strains of B. pertussis may be employed with other strains of

Bordetella, such as B. parapertussis, B. bronchiseptica and B. avium. The technique also broadly is applicable to hybrid genes formed from any structural gene coding for a desired gene product and any autologous promoter for another structural gene to effect expression of the desired gene product from an organism transformed by the hybrid gene.

Accordingly, in yet another aspect of the present invention, there is provided a method of expression of a gene product from an organism, which comprises forming a hybrid gene comprising a structural gene coding for a desired gene product fused to a promoter for another structural gene, transforming the organism with the hybrid gene, and culturing the transformed organism to effect expression of the gene product from the transformed organism.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the sequence of the DNA cassette used to link the FHA promoter and TOX structural gene sequences, the arrow indicating the location of the ATG start codon of the SI gene. The plasmid S-3680-10 contains the hybrid operon sandwiched between the flanking regions of TOX and was used for introduction into a TOX deleted strain of B. pertussis;

Figure 2 shows the plasmid S-3749-18 which contains the hybrid FHAp/TOX operon sandwiched between the flanking regions of FHA, used for introduction into an FHA deleted strain of B. pertussis;

Figure 3 shows the kinetics and expression levels of PT from strains (A) 10536, (B) 390-59, and (C) 590-11. Strain 390-59 contains the hybrid FHAp/TOX operon at the TOX locus while strain 590-11 contains FHAp/TOX operon at the FHA locus plus the native TOX operon at the TOX locus;

Figure 4 shows the sequence of the DNA cassette used to link the TOX promoter and FHA structural gene, the arrow indicating the ATG codon of the FHA structural gene. The plasmid S-3838-9 contains the hybrid operon sandwiched between the FHA flanking regions for delivery to the FHA locus of an FHA deleted strain;

Figure 5 shows the kinetics and expression of FHA from strains (A) 10536 and (B) 890-49 which contains the TOXp/FHA hybrid gene at the FHA locus;

Figure 6 shows the sequence of the DNA cassette used to link the FHA promoter and pertactin structural g ne, the arrow indicating the ATG codon of the pertactin gene. The plasmid S-3982-7 contains the hybrid gene betw en the p rtactin flanking regions for delivery to the pertactin locus of a PRN deleted strain;

Figure 7 shows the kinetics and expression of the 69 kDa protein from strains (A) 10536 and (B) 1290-4 which contains the FHAp/PRN hybrid at the PRN locus;

Figure 8 shows the Southern blot of restricted genomic DNA obtained from B. pertussis strains 10536 and 390-59, which contains the FHAp/TOX hybrid at the TOX locus, and indicates that the hybrid operon was correctly placed;

Figure 9 shows the Southern blot analysis of strains 10536 and 890-49, which contains the TOXp/FHA hybrid at the FHA locus, and indicates that the hybrid operon was correctly placed; and

Figure 10 shows the Southern blot analysis of strains 10536 and 1290-4, which contains the FHAp/PRN hybrid at the PRN locus, and indicates that the hybrid operon was correctly placed.

GENERAL DESCRIPTION OF INVENTION

Bordetella pertussis 10536 is the vaccine production strain of the assignee hereof and it has been used as the initial strain for all the work detailed by the inventors herein. The genes for PT, FHA, and pertactin have been cloned and sequenced (Nicosia et al., Proc. Natl. Acad. Sci., U.S.A., 83, 4631, [1986]; Loosmore et al., Nucl. Acids Res., 17, 8365, [1989] Relman et al., Proc. Natl. Acad. Sci., U.S.A., 86, 2637, [1989]; Charles et al., Proc. Natl. Acad. Sci., U.S.A., 86, 3554, [1989]).) and the promoter regions and transcriptional start of the structural genes have been determin d. The inventors have generated hybrid genes by substituting the native promoters of a given gene by promoters from another gene of the organism. This was accomplished by fusing the promoters with the structural genes at the ATG start codon of the structural gene. Such fusions result in a native but autologous promoter, and a structural gene with its natural signal sequence. The resultant hybrid genes th n have been integrated at the appropriate gene loci in the chromosome of B. pertussis by homologous recombination.

As examples of the use of autologous promoters, genes have been created containing an FHA promoter with the TOX operon (FHAp/TOX), an FHA promoter with the PRN gene (FHAp/PRN), and the TOX promoter with the FHA gene (TOXp/FHA). A number of B. pertussis strains have been generated to demonstrate the efficacy of this strategy. In all cases, the autologous, promoter functioned at its new location on the genome.

It was clearly demonstrated by this work that the promoter of a structural gene may be replaced by that of another gene, by inserting th FHAp/TOX hybrid at the TOX locus to generate B. pertussis strain 390-59. PT was secreted by this recombinant strain with kinetics and yields comparable to the wild-type strain 10536. (see Figure 3B in comparison to normal kinetics of strain 10536 shown in Figure 3A). The

FHAp/TOX hybrid then was directed to the native FHA locus to generate B. pertussis strain 590-11 which now contains two copies of TOX. The yield and kinetics of PT production were consistant with the expression from two TOX genes, one directed by the FHA promoter in the hybrid operon, and the other from the native operon (see Figure 3C). This experiment demonstrated that the FHAp/TOX hybrid gene may be integrated at two different loci in the genome.

PT holotoxin is an oligomeric protein composed of six subunits encoded by five different genes. In order to determine whether the FHA promoter can direct the expression of an increased amount of protein if the protein were not as structurally complex, a strain was generated containing the FHAp/PRN hybrid gene (B. pertussis strain 1290-4). The pertactin protein is a single polypeptide produced from a larger precursor. The yield of pertactin was increased by three-fold in fermenters and ten-fold in shake flasks when its synthesis was directed by the FHA promoter (see Figure 7B, with the normal kinetics of pertactin production in a 10-litre fermenter shown in Figure 7A).

The overproduction of FHA can pose a problem in certain purification protocols. In order to reduce the yield of FHA to facilitate the purification of other antigens procuced in lesser amounts, the native promoter was substituted by the TOX promoter. The TOXp/FHA hybrid gene was directed to the FHA locus (B. pertussis strain 890-49) and the amount of FHA produced was significantly reduced, 50 to 100-fold (see Figure 5B, with normal kinetics of FHA production shown in Figure 5A).

The inventors have demonstrated by this work, as detailed in the Examples below, that it is possible to substitute promoters between two Bordetella genes and obtain antigen production from the substituted promoters. It has been further demonstrated that it is possible to increase or decrease the yield of antigens in Bordetella pertussis by promoter replacement. The production of pertactin has been increased and that of FHA decreased. Although no significant changes were observed in the kinetics of antigen production, such a promoter replacement strategy also may be used to alter the time-course as well as the yield of production of certain antigens. The regulation of protein production by this approach has broad application for fermentation and downstream processing technologies. Such a promoter replacement strategy can be used with any combination of genes in any genus of the species Bordetella and may be applied to other organisms.

EXAMPLES

Methods of m lecular genetics, fermentation, protein biochemistry, and hybridoma technology used but not explicitly described in this disclosure and thes Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art. Further techniques of manipulation of B. pertussis are described in published European Patent Application Publication No. 0,322,115; U.S. patent application Serial No. 275,376 filed September 28, 1990, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference.

All oligonucleotides were synthesized on an ABI model 380A DNA synthesizer and were purified by polycrylamide gel electrophoresis. DNA manipulations were according to Sambrook et al. (Molecular cloning: a laboratory manual, second edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. [1989]). Restriction enzymes were used according to manifacturers' specifications. The TOX, FHA, and PRN genes were cloned from a lambda Charon 35 library containing Sau3A I fragments of pertussis DNA from strain 10536. The structures of the chromosomal genes were confirmed by restriction mapping and partial or complete gene sequencing. Promoters of given genes were linked to the coding sequences of other genes using synthetic oligonucleotides. The correct fusions between the promoters and structural genes were confirmed by dideoxy DNA sequencing (Sanger et al., Proc. Natl. Acad. Sci., U.S.A. 74, 5463, [1977]). Gene replacement in B. pertussis was achieved by homologous recombination following introduction of plasmid DNA into the cell by electroporation (Zealey et al., Bio/Technology, 8, 1025, [1990]). The TOX-deleted strain 29-8, FHA-deleted strain 390-101, and PRN-deleted strain 1090-108-3 were all prepared in a similar manner. In some instances, genes were integrated as non-replicating plasmids and allelic replacement achieved by growth of primary transformants under streptomycin selection (Stibitz et al., Gene, 50, 133, [1986]). Chromosomal DNA was prepared as described by Yacoob and Zealey (Nucl. Acids Res. 16, 1639, [1988]) and Southern blot analysis performed according to Sambrook et al. using Gene Screen Plus (Dupont).

Example 1:

This Example illustrates the construction of the FHAp/TOX hybrid operon and its introduction into B. pertussis 10536.

A pUC-based plasmid (S-3680-10) was constructed which contains the FHA promoter directing the expression of the native pertussis toxin structural gene and surrounded by the TOX flanking regions. The FHA promoter is an EcoR I/Hinf I fragment of approximately 240 bp which includes any Bvg responsive elements (the TOX operon requires 170 bp for the Bvg responsive region). The complimentary oligonucleotides bridging the two gene sequences were annealed as an approximately 45 bp Hinf I/Ava I cassette which includes th start codon of the DNA segment coding for the leader sequence of PT subunit

S1 (see Figure 1). The remainder of the TOX operon from the Ava I site of the S1 gene to the EcoR I following the end of translation was used to complete the hybrid operon. The 3 kb Sma I/EcoR I 5'-flanking region and 4 kb EcoR I/Sal I 3'-flanking sequence of the native TOX locus were used to direct the hybrid operon to the TOX locus. (Figure 1).

The FHAp/TOX hybrid plasmid was introduced into a TOX deleted derivative of B. pertussis 10536 (strain 29-8) by electroporation and this generated a strain (390-59) in which PT expression is directed by the FHA promoter. The kinetics and yield of PT expression from strain 390-59 were equivalent to those obtained with the parent strain 10536 (Figures 3A and 3B). The correct in situ integration of the FHAp/TOX hybrid operon at the TOX locus was demonstrated by restriction mapping and Southern blot analysis as shown in Figure 8. The TOX deleted strain 29-8 has been deposited with the ATCC (accession number 53973) and is described in the aforementioned USSN 275,376.

Example 2:

This Example illustrates the construction of the FHAp/TOX hybrid operon and its introduction into an FHA-deleted strain of B. pertussis.

A pBR322-based plasmid (S-3749-18) was constructed which contains the FHA promoter fused to the structural genes for TOX at the start codon of the DNA segment coding for the S1 subunit leader sequence, surrounded by the FHA flanking regions. The hybrid operon was constructed as in Example 1. A 2.5 kb Bgl II/EcoR I 5'-flanking and a 1.7 kb EcoR I/Cla I 3'-flanking regions were used to direct the hybrid operon to the FHA locus (Figure 2).

This FHAp/TOX hybrid plasmid was introduced into the FHA-deleted strain (390-101). This generated a strain (590-11) with two copies of TOX, one transcribed by its native promoter and one by the FHA promoter. The overall yield of PT was about twice that of strain 10536 and the kinetics of PT production were unchanged (see Figures 3A and 3B). The FHA-deleted strain 390-101 has been deposited with the ATCC on March 6, 1991 (accession number ATCC 55157).

Example 3:

This Example illustrates the construction of the TOXp/FHA hybrid gene and its introduction into B. pertussis.

A pBR322-based plasmid (S-3838-9) was constructed which contains an approximately 500 bp f the TOX promot r region, starting at the EcoR I site. This sequence includes the Bvg responsive region at -170 bp. The 460 bp EcoR I/Nco I fragment of the TOX promoter is bridged to the FHA structural gene through an -100 bp Nco I/Sph I oligonucleotide (Figure 4). The remainder of the FHA B structural gene from the Sph I site to the EcoR I site completed the hybrid operon. The 2.5 kb Bgl II/EcoR I site FHA 5'-(flanking and 1.7 kb EcoR I/Cla I 3'-flanking regions directed the hybrid operon to the FHA locus (Figure 4). The correct integration of the TOXp/FHA hybrid operon at the FHA locus was demonstrated by restriction mapping and Southern blot analysis as shown in Figure 9.

The strain (890-49) resulting from the chromosomal integration of the hybrid operon produced significantly reduced levels of FHA (Figures 5A and 5B).

Example 4:

This example illustrates the construction of th FHAp/PRN hybrid gene and its introduction into B. pertussis.

The pUC-based plasmid S-3982-7 contains the FHA promoter fused at the start codon for the signal sequence of the pertactin structural gene and surrounded by PRN flanking sequences. The FHA promoter is an approximately 240 bp EcoR I/Hinf I fragment which is fused to the PRN structural gene through a 93 bp Hinf I/Nco I oligonucleotide (Figure 6). The remainder of the pertactin structural gene from the Nco I site was added to complete the hybrid gene. The 1.6 kb Sau3A I/EcoR I 5'-flanking and 8 kb Apa I/Sau3A I 3'-flanking regions from PRN directed the hybrid gene to the PRN locus (see Figure 6). The correct in situ placement of the FHAp/PRN hybrid gene at the PRN locus of the PRN deleted strain 1090-108-3 was demonstrated by restriction mapping and Southern blot analysis in Figure 10.

Strain 1290-4 containing the FHAp/PRN hybrid produced enhanced amounts of the pertactin outer membrane protein (see Figures 7A and 7B). The PRN deleted strain 1090-108-3 and strain 1290-4 have been deposited with the ATCC on March 6, 1991 (accession numbers ATCC 55156 and ATCC 55155, respectively).

Example 5:

This Example illustrates the preparation of B. pertussis strains containing hybrid operons and their structural analysis.

Strains deleted for TOX (29-8), FHA (390-101), or PRN (1090-108-3) were engineered using homologous recombination between the Connaught vaccine strain 10536 and plasmid DNA containing flanking regions of the desired genes, as described by Zealey t al. (Bio/Technology 8, 1025, [1990]). The flanking regions used for deleting and replacing g nes were as describ d in Examples 1, 2, 3 and 4. The flanking regions surrounded a cassette containing a tetracycline resistance gene which was inserted at th locus for

the deleted structural genes. During another round of allelic exchange, this cassette was subsequently replaced by the hybrid genes.

To confirm correct in situ placement of the hybrid genes, restriction digests and Southern blot analyses were performed on genomic DNA purified from the recombinant strains.

Example 6:

This Example illustrates the preparation of Southern blots for the B. pertussis strain 390-59 (see Figure 8).

Chromosomal DNA was isolated from wild-type (WT) B. pertussis 10536 (lanes 1-2) and B. pertussis 390-59 (lanes 3-4), restricted with the endonucleases Kpn I/Sma I (lanes 1 and 3) and Bgl II (lanes 2 and 4) and probed with a 4.7 kb EcoR I restriction fragment that represents the entire TOX coding sequence. Replacement of the TOX promoter by the FHA promoter resulted in the loss of a KpnI site as shown by the arrow on Figure 8. Digestion with KpnI and SmaI produced TOX-specific hybridization fragments of 3.0, 1.6 and 5.1 kb for B. pertussis 10536 and 4.8 and 5.1 for B. pertussis 390-59.

Example 7:

This Example illustrates the preparation of a Southern blot for B. pertussis strain 890-49 (Figure 9).

Chromosomal DNA was isolated from wild-type (WT) B. pertussis 10536 (lanes 1-2) and B. pertussis 890-49 (lanes 3-4), restricted with the endonucleases Kpn I/Bgl II (lanes 1 and 3) and Bam HI (lanes 2 and 4) and probed with an FHA specific probe. Replacement of the FHA promoter by the TOX promoter resulted in the introduction of a Kpn I site as shown in Figure 9. Digestion with Kpn I and Bgl II produced FHA-specific hybridizaion fragments of 9 kb for B. pertussis 10536 and 6 kb for B. pertussis 890-49.

Example 8:

This Example illustrates the preparation of a Southern blot for B. pertussis strain 1290-4 (see Figure 10).

Chromosomal DNA was isolated from wild-type (WT) B. pertussis 10536 (lanes 1-2) and B. pertussis 1290-4 (lanes 3-4), restricted with the endonucleases Apa I (lanes 1 and 3) and Sal I (lanes 2 and 4) and probed with a PRN specific probe. Replacement of the PRN promoter by the FHA promoter resulted in the introduction of an ApaI site which produced PRN-specific hybridization fragments of 4.4 kb for B. pertussis 10536 and 2.5 kb for B. pertussis 1290-4 as shown in Figure 10.

Example 9:

This Example illustrates the growth of B. pertussis strains and immunoasays for the quantification of specific antigens.

Bordetella pertussis strains were grown in modified Stainer-Scholte medium containing 0.2% heptakis (2,6-0-dimethyl)β-cyclodextrin (Imaizumi et al, Infect. Immun. 41, 1138, [1983]) either in 10 litre ChemAp fermenters, controlled for pH and dissolved oxygen, or in 10 ml culture. Culture supernatants were tested for antigen production in antigen-specific ELISAs. PT was measured in a fetuin-capture ELISA as described in Loosmore et al. (Infect. and Immun. 58, 3653, [1990]). FHA was captured by a mouse monoclonal anti-FHA antibody purified from ascites fluid and the second antibody was a polyclonal rabbit IgG anti-FHA conjugated to horse-radish peroxidase. Pertactin was captured with a polyclonal antibody purified from a monospecific guinea pig hyperimmune serum and the second antibody was a guinea pig IgG anti-69 kDa conjugated to horse-radish peroxidase. PT, FHA and 69 kDa proteins purified from fermentation broths of the wild-type production strain 10536 were used as standards.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a novel gene product expression method having particular application to Bordetella species. Modifications are possible within the scope of this invention.

Claims

1. A hybrid pertussis gene, characterized by a structurel pertussis gene fused at an ATG start codon to a native but aurologous pertussis promoter.

2. The gene claimed in claim 1 wherein said promoter is the TOX promoter and the structural pertussis gene is the FHA or PRN gene, said promoter is the FHA promoter and the structural pertussis gene is the TOX or PRN gene, or said promoter is the PRN promoter and the structural pertussis gene is the TOX or FHA gene.

3. A strain of Bordetella characterized by being transformed by a hybrid pertussis gene as claimed in claim 1 or 2, or a multiple of said hybrid gene.

4. The strain claimed in claim 3 wherein the strain of Bordetella transformed by said hybrid pertussis gene is Bordetella pertussis.

5. The strain claimed in claim 4 wherein said transformed strain produces, upon culture of said transformed strain, said gene product at a yield of production which is altered from the yield of production achieved by the same Bordetella strain transformed by a homologous gene comprising a structural pertussis gene fused at an ATG start codon to its own native pertussis promoter.

6. The FHAp/PRN transformed PRN$^-$ strain of B. pertussis No. 1290-4, as deposited with ATCC under accession No. 55155, or the FHAp/TOX (TOX) transformed TOX$^-$ strain of B. pertussis No. 390-59, or the FHAp/TOX (FHA) transformed FHA$^-$ strain of B. pertussis No. 590-11 or the TOXp/FHA (FHA) transformed FHA$^-$ strain of B. pertussis No. 890-49.

7. The FHA$^-$ stain of Bordetella pertussis No. 390-101 having ATCC accession No. 55157, or the PRN$^-$ strain of Bordetella pertussis No. 1090-108-3 having ATCC accession No. 55156.

8. Plasmid S-3680-10 comprising FHAp/TOX and TOX flanks having the restriction map shown in Figure 1, or plasmid S-3749-18 comprising FHAp/TOX and FHA flanks having the restriction map shown in Figure 2, or plasmid S-3838-9 comprising TOXp/FHA with FHA flanks having the restriction map shown in Figure 4, or plasmid S-3982-7 comprising FHAp/PRN flanks having the restriction map shown in Figure 6.

9. An antigenic protein produced as the gene product from the transformed strain of Bordetella claimed in claim 3.

10. The antigenic protein claimed in claim 9 which is pertussis toxin (PT), filamentous haemagglutinin (FHA) or pertactin (69 kDa).

11. A component vaccine against Bordetella pertussis comprising at least an effective amount of an antigenic protein as claimed in claim 9 and a physiologically acceptable-carrier therefor.

12. A whole cell vaccine against Bordetella pertussis comprising a killed form of a transformed strain of Bordetella pertussis as claimed in claim 4 and a physiologically-acceptable carrier therefor.

13. A method of expression of a gene product from an organism, characterized by (a) transforming said organism with a gene coding for a d sired g ne product, and (b) culturing said transformed organism to effect expression of said gene product from said transformed organism, characterized by effecting said transforming step with a hybrid gene comprising a structural gene coding for a desired gene product fused to a promoter for another structural gene.

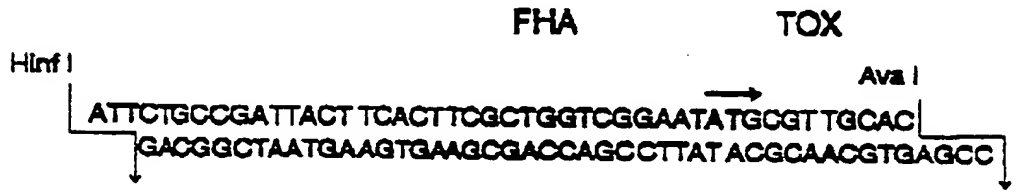
14. The method claimed in claim 13, wherein said expression of said gene product is effected at a production yield which is different from the production yield achieved wherein said organism is transformed by a homologous gene comprising said structural gene and its own native promoter.

15. The method claimed in claim 13 or 14, wherein said expression of said gene product is effected with synchronicity or asynchronocity of production.

16. The method claimed in claim 14, 15 or 16, wherein transformation of said organism is effected by integrating said hybrid gene at the appropriate gene loci in the chromosome of the organism by homologous recombination.

17. The method of claimed in any one of claims 14 to 16, wherein said organism is Bordetella pertussis.

Figure 1. Map of the vector used to integrate the hybrid FHAp/TOX operon at the TOX locus



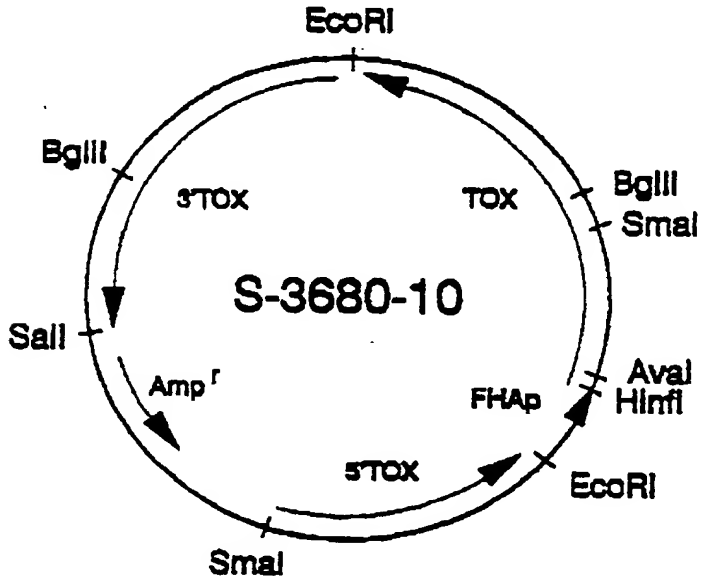


Figure 2. Map of the vector used to integrate the hybrid FHAp/TOX operon at the FHA locus

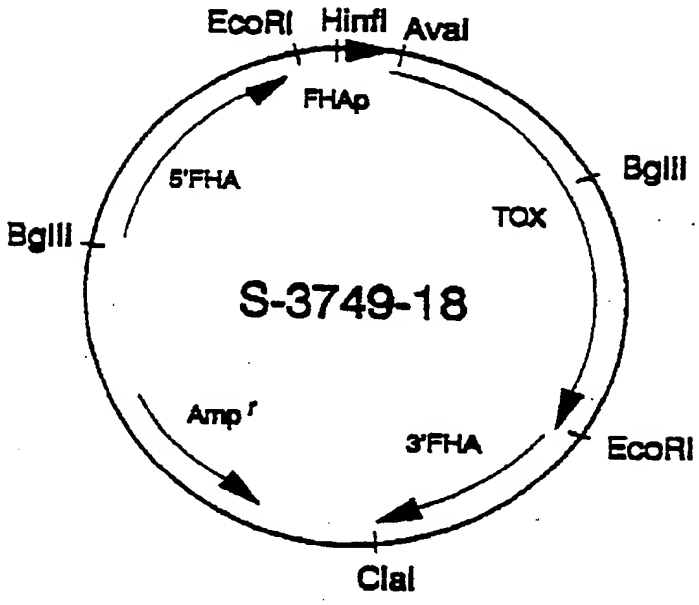
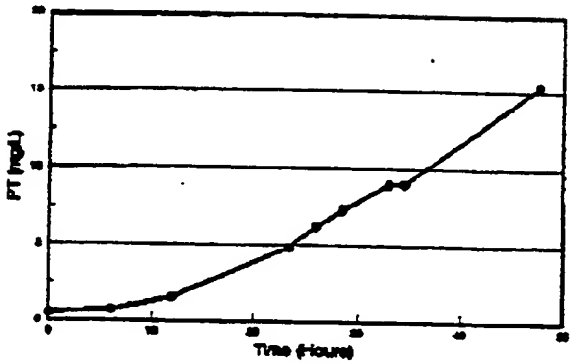
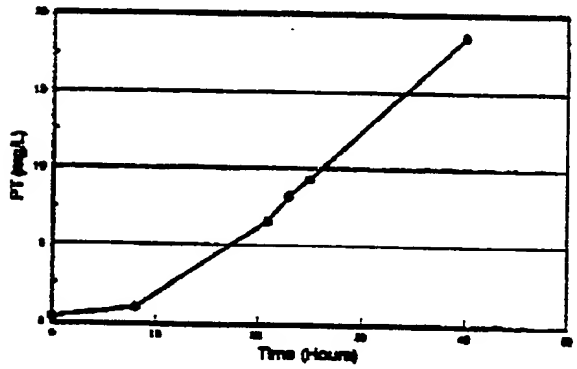


Figure 3.

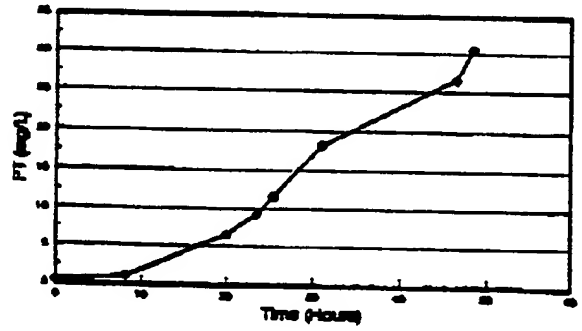
A.



B.

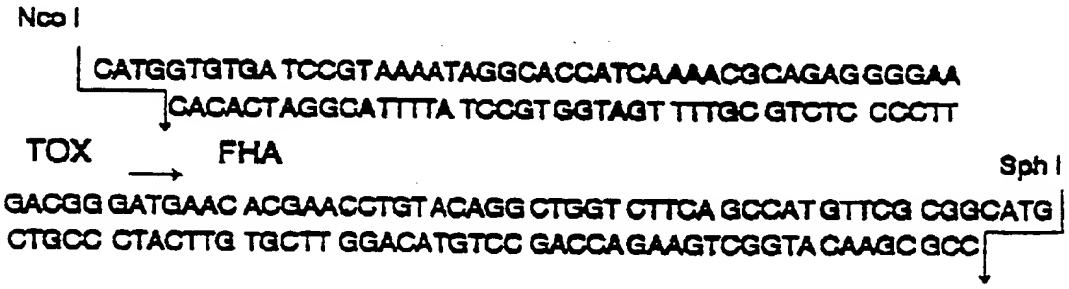


C.



PT (mg/L)

Figure 4. Map of the vector used to integrate the the hybrid TOXp/FHA gene at the FHA locus



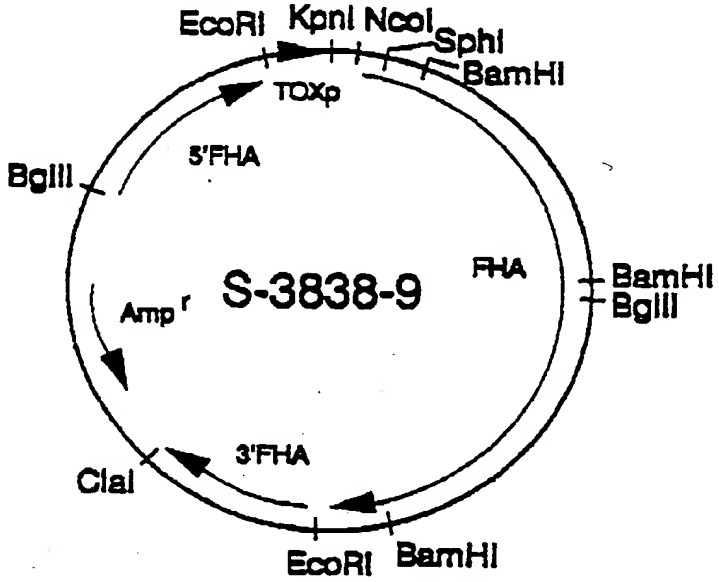
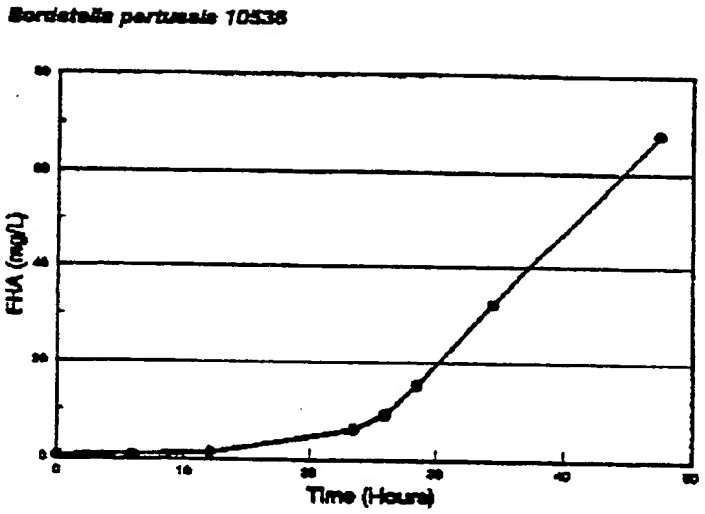
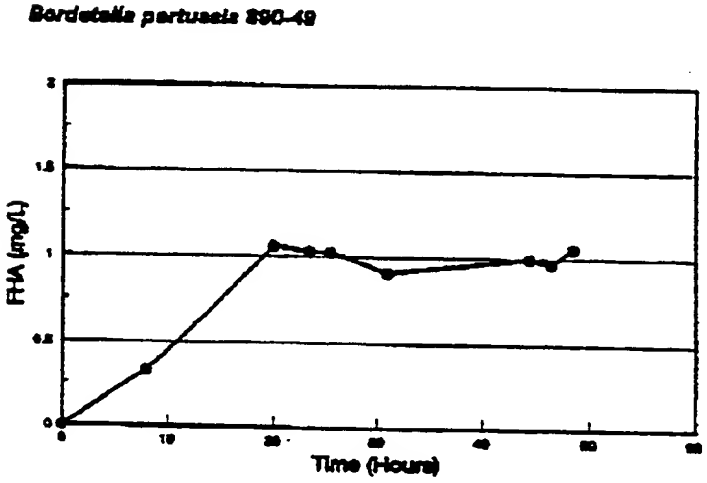


Figure 5.

A.

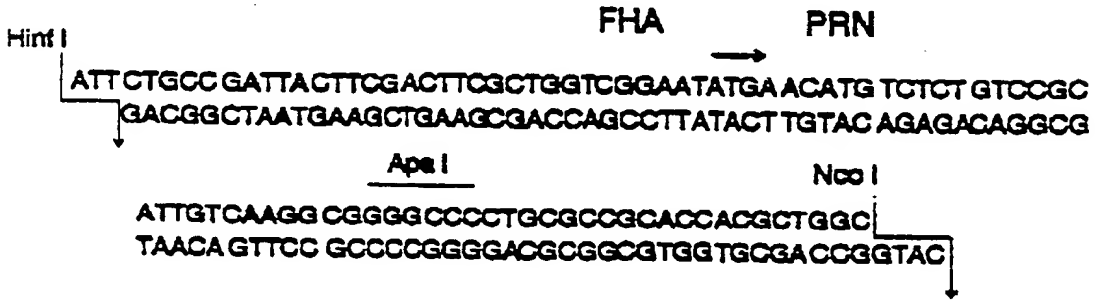


B.



FHA (mg/L)

Figure 6. Map of vector used to integrate the hybrid FHAp/PRN gene at the PRN locus



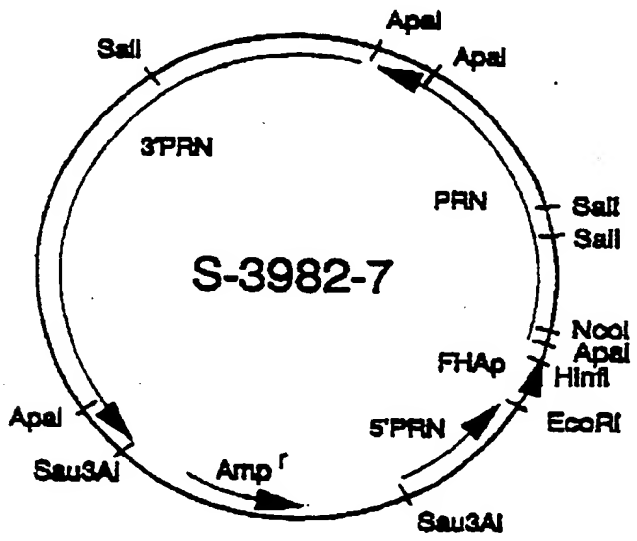
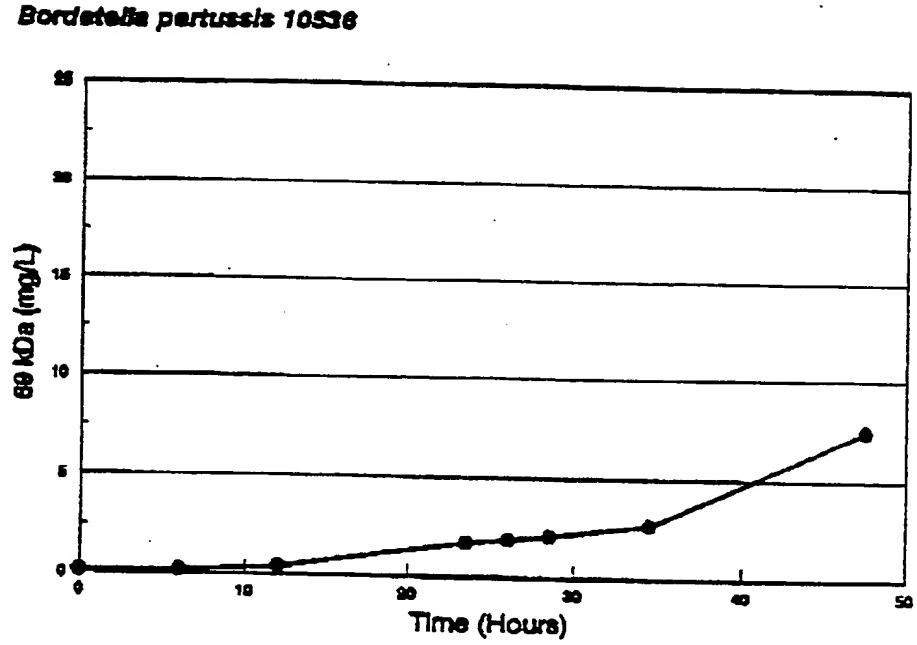


Figure 7.

A.



B.

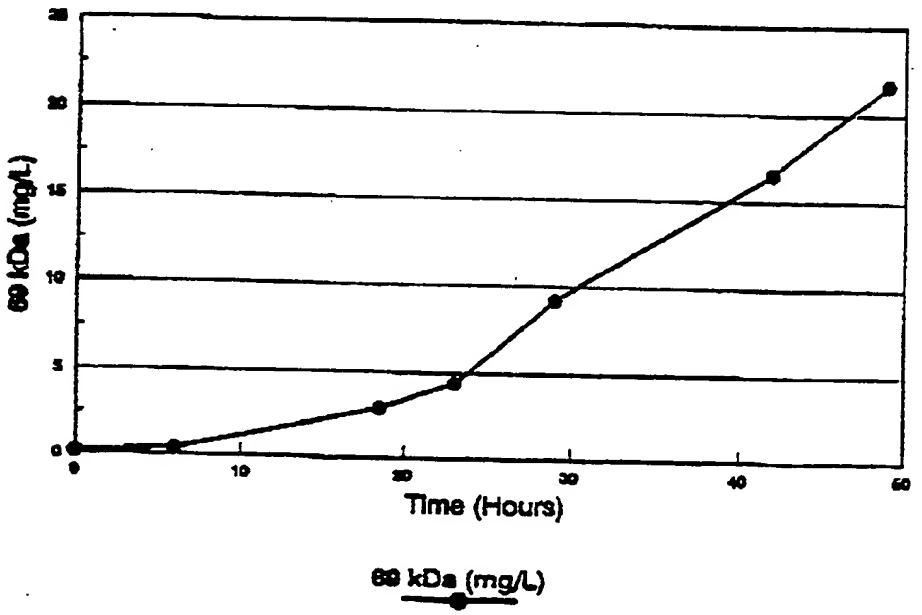


Figure 8. Southern analysis of recombinant Bordetella pertussis strain 390-59 containing the FHAp/TOX operon at the TOX locus

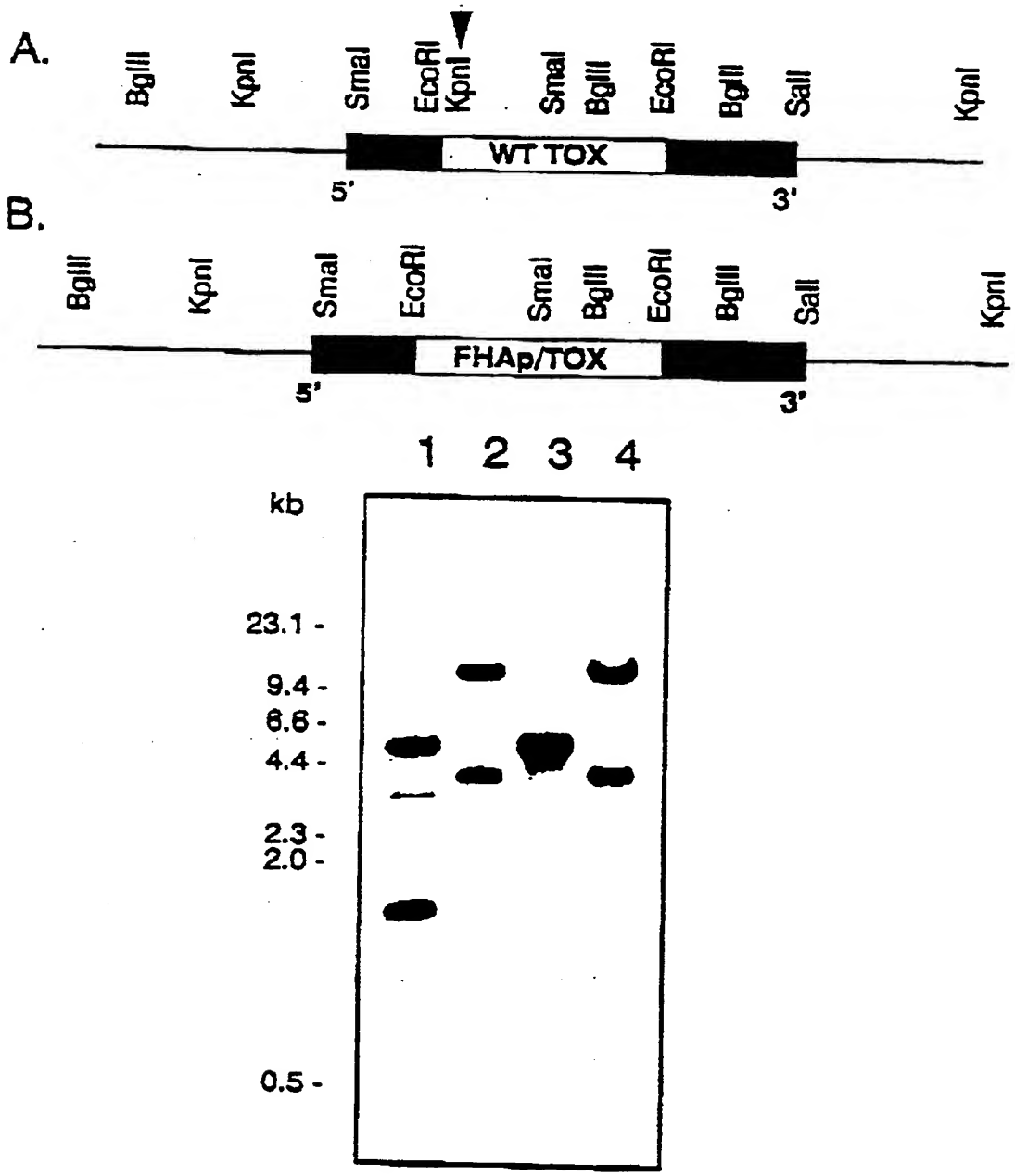


Figure 9. Southern analysis of recombinant Bordetella pertussis strain 890-49 containing the TOXp/FHA gene at the FHA locus

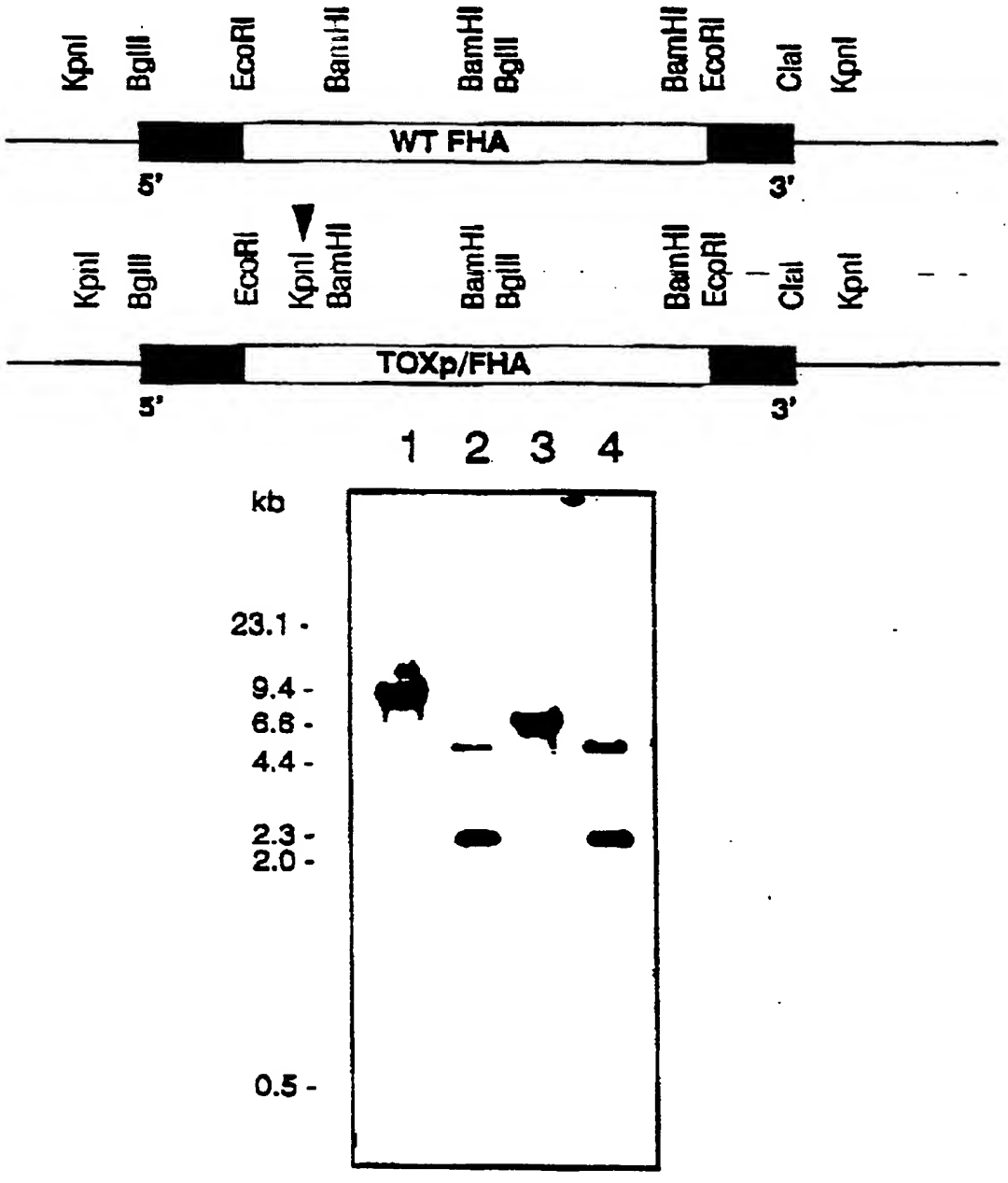


Figure 10. Southern analysis of recombinant Bordetella pertussis strain 1290-4 containing the FHAp/PRN gene at the PRN locus